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PRELIMINARY DATA

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Effects of acidity on cell division (H. 2)

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It has been reported that carbon dioxide may act as a powerful inhibitor of cell division (2). If synchronized 7-hour old cells of *Chlorella* 7-11-05 are resuspended in distilled water, the division readily proceeds in dark in atmospheric air but not in air enriched with carbon dioxide. The inhibitory action of carbon dioxide is effected by other ions. Thus, in bicarbonate buffers, a molar concentration of bicarbonate equal to or higher than that of carbon dioxide in the suspending fluid completely suppresses the adverse action of carbon dioxide on cell division. The balancing action of bicarbonate extends up to  $1 \times 10^{-2} \text{ M CO}_2$ . At higher concentrations of carbon dioxide, bicarbonate is unable to suppress  $\text{CO}_2$  inhibition of cell division. Other acids act similarly on cell division. So far the effects of hydrochloric, nitric, sulfuric, phosphoric, acetic, glycolic, and citric acids have been studied.

In these experiments, synchronized cells of the green, high-temperature alga, *Chlorella* 7-11-05 (1), were grown under optimal

conditions. After 7 hours in light, cells were centrifuged and transferred into a suspending fluid containing a certain concentration of acid. Both buffered and unbuffered solutions were used. Cell suspensions were then placed in darkness and supplied through bubbling tubes either with atmospheric air or with gas mixtures containing different percentages of carbon dioxide in atmospheric air.

Cell division was measured by counting undivided cells. This number was then subtracted from the original number of mother cells in a given volume of cell suspension and the difference was expressed in per cent of the original number of cells. The results of three series of experiments, based on observations on cells kept under given conditions in darkness for 16 hours, are presented in table 1.

In one series of experiments, cells were suspended in distilled water or in  $1 \times 10^{-5}$  N and  $1 \times 10^{-4}$  N sulfuric acid. The suspending fluid was unbuffered and the cell suspension was supplied during division time in darkness with atmospheric air. Cell division proceeded satisfactorily in distilled water and in  $1 \times 10^{-5}$  N  $H_2SO_4$ , but not in  $1 \times 10^{-4}$  N  $H_2SO_4$ , which proved to be inhibitory to cell division.

In the second series of experiments, the suspending fluid was buffered with  $1 \times 10^{-2}$  N Tris(hydroxymethyl)aminomethane buffer and supplied during division time, as in series one, with atmospheric air. Here the division took place in a wide range of concentrations of sulfuric acid up to  $1 \times 10^{-4}$  N  $H_2SO_4$ . The adverse effect of sulfuric acid was cancelled by the buffer. However, an increase in  $H_2SO_4$  concentration by  $1 \times 10^{-4}$  N tipped the balance between  $H_2SO_4$  and "Tris" and caused inhibition of cell division.

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In the third series of experiments, cells were supplied in dark with 1 per cent  $\text{CO}_2$ -in air mixture. Cells suspended in distilled water did not divide in the absence of buffer. Thus, one per cent  $\text{CO}_2$  ( $2.4 \times 10^{-4} \text{ N}$ ) proved to be inhibitory to cell division. The percentage of cells divided in  $1 \times 10^{-2} \text{ N}$  and in  $1.01 \times 10^{-2} \text{ N H}_2\text{SO}_4$  was lower than in atmospheric air (Series II), indicating that the effect of carbon dioxide was added to that of sulfuric acid.

For a given batch of cells, the inhibitory acid concentration has a precisely determinable value. However, in a series of experiments it becomes evident that the acidic action on cell division is affected by other factors which are under study in our laboratory.

#### REFERENCES

1. Serokin, C., Nature, London, 184, 613 (1959).
2. Serokin, C., Nature, London, In Press.

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1. This work was supported by funds from the National Aeronautics and Space Administration.
2. Scientific Article A974, Contribution No. 1356 of the University of Maryland Agricultural Experiment Station.

Table 1. Cell division in synchronized 7-hour cells of *Chlamydomonas reinhardtii* after 16 hours in darkness at 35°.

Series	Sustaining		Gas	Per cent cell division
	fluid	Buffer		
I	H <sub>2</sub> O	No	Atmospheric air	0
	1 x 10 <sup>-5</sup> M H <sub>2</sub> SO <sub>4</sub>	"	"	25
	1 x 10 <sup>-4</sup> "	"	"	5
II	H <sub>2</sub> O	1 x 10 <sup>-2</sup> M Tris	"	35
	1 x 10 <sup>-2</sup> M H <sub>2</sub> SO <sub>4</sub>	"	"	30
	1.01 x 10 <sup>-2</sup> "	"	"	10
III	H <sub>2</sub> O	No	100% CO <sub>2</sub> in air	0
	H <sub>2</sub> O	1 x 10 <sup>-2</sup> M Tris	"	0
	1 x 10 <sup>-2</sup> M H <sub>2</sub> SO <sub>4</sub>	"	"	0
	1.01 x 10 <sup>-2</sup> "	"	"	0